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WORKSHOP PROCEEDINGS: APPROACHES FOR IMPROVING THE ASSESSMENT OF HUMAN GENETIC RISK--HUMAN BIOMONITORING

December 13-15, 1982
Washington, DC

by

John R. Fowle III*
Reproductive Effects Assessment Group
Office of Health and Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC 20460

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*Workshop participants are listed on page viii. They all contributed to the preparation of this report.

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PREFACE

This report explores ways to improve the ability to predict genetic risk to humans. Certain Federal laws administered by EPA require that a pollutant's negative impacts on society be weighed against its benefits in arriving at regulatory decisions. To accomplish this one must somehow quantify the risks and benefits for comparative purposes. When cause and effect relationships can be demonstrated in humans after exposure to a toxicant, quantifying risks and benefits is a straight-forward process. But for genetic diseases, chronic effects observed in the offspring of exposed individuals, it is very difficult and requires many assumptions. There are no data linking pollutant exposures to the induction of heritable mutations in humans, although this has been demonstrated in experimental studies using mice. Furthermore, even though data can be obtained from exposed humans showing that adverse genetic effects are occurring, these can only be measured in somatic tissue, not germinal tissue. Thus, in order to arrive at estimates of the risk of heritable genetic diseases in humans after exposures to mutagens, data on heritable effects from experimental rodent systems and/or data from humans on somatic cell effects must be somehow extrapolated to heritable effects likely to occur in humans. This requires assumptions about species-to-species relationships and tissue-to-tissue relationships. Furthermore, high- to low-dose extrapolations are usually invoked when using the experimental data because such studies are usually conducted at much higher doses than humans are exposed to in the environment.

Because of these uncertainties, a workshop was held to examine the assumptions implicit in making quantitative estimations of heritable human mutation frequencies using human and animal data. Emphasis was placed on ways to assess the correctness of some of the assumptions and ways to verify the risk assessment approaches.

In so doing it was pointed out that the experimental animal data must be anchored to comparable human data. Recommendations were made to study somatic and heritable damage in individuals from high risk human populations (e.g., cancer chemotherapy patients) and to study the corresponding effects in parallel experiments using mice receiving the same treatment.

It is believed that the information in this report will be particularly useful to risk assessors, genetic toxicologists, epidemiologists, and research planners. It is hoped that the report will stimulate collaborative efforts among government agencies, and among government agencies and other organizations as well. Continued examination and refinement of risk assessment approaches will provide a mechanism for more accurate prediction of human genetic risk. It is hoped that the efforts described in this report will serve as a springboard for generating a firm data base to support future risk assessment efforts.

Because of the dynamic nature of this subject, this report cannot be considered comprehensive or complete. New questions continue to arise about the assessment of mutagenic risk as chemical interactions with cellular macromolecules (such as DNA) become better known and as interspecies and intertissue relationships begin to unravel. This report provides a reasonable outline of the state-of-the-subject in 1983. It should be considered a guide for planning work, hopefully, to develop a scientifically credible approach for using human biomonitoring data for genetic risk assessment.

ABSTRACT

Federal laws require a consideration of adverse health effects, including mutagenicity, in arriving at regulatory decisions on chemical substances. Certain laws require balancing the consequences of these risks with the benefits provided by the use of chemical substances. This requires that risk be quantitatively assessed. Estimates of human genetic risk can be made indirectly based on data from animal experimentation and human somatic cells, but it is not practical to estimate genetic risk directly based on data from human germ cells. The indirect estimates are highly debated because of uncertainties about interspecies and interorgan extrapolations. Uncertainties in extrapolating from effects observed in animals at high experimental doses to effects likely to occur in humans at much lower environmental levels further complicate genetic risk assessment. Comparative studies are needed to define the relationships between somatic cell and germ cell events and between experimental animals and humans. This may involve selecting at least one high risk human population for study. These efforts will require a long-term coordination of efforts among the Federal agencies and among government agencies, industrial concerns, and the academic community.

PARTICIPANTS AND REVIEWERS

Organizing Committee

John R. Fowle III, Co-Project Officer
U.S. Environmental Protection Agency

Raymond R. Tice, Co-Project Officer
Brookhaven National Laboratory

Vicki L. Vaughan-Dellarco
U.S. Environmental Protection Agency

Ernest R. Jackson
U.S. Environmental Protection Agency

Other Participants

K.S. Lavappa
U.S. Environmental Protection Agency

Sheila L. Rosenthal
U.S. Environmental Protection Agency

Michael D. Waters
U.S. Environmental Protection Agency

Speakers/Participants

Richard J. Albertini
University of Vermont

J. Grant Brewen
Allied Chemical Co.

Gerald L. Chan
Dana-Faber Cancer Institute

James E. Cleaver
University of California
San Francisco

Roger W. Giese
Northeastern University

George R. Hoffman
Holy Cross College

James V. Neel
University of Michigan

James A. Swenberg
Chemical Industry Institute of
Toxicology

Lawrence R. Valcovic
U.S. Food and Drug Administration

I. INTRODUCTION

A considerable genetic disease burden has been recognized in the human population. It is estimated that perhaps 10% of all human disease has a significant genetic component, resulting from changes in the composition, arrangement, or number of genes and chromosomes (BEIR 1980, Flamm 1977, UNSCEAR 1977). Humans are exposed to a large and increasing number of chemical substances, some of which have mutagenic effects in other organisms and may pose a genetic risk to people. Because induced genetic diseases can only be expressed in future generations, much effort has gone into designing methods for detecting mutagenic agents. Recently, combinations of tests that are quite effective at identifying mutagenic chemicals have been developed. However, these tests are not useful in monitoring humans for heritable mutations, and thus, the magnitude of the contribution that chemical mutagens may make to human genetic disease is highly debated. Despite the lack of definitive evidence, there is no reason to doubt that chemical mutagens can induce heritable, germ-line mutations in human beings.

Concerns about the ability of man-made chemical substances to alter the environment led to the passage of Federal laws to protect against such effects. All of these laws require a consideration of adverse health effects in arriving at regulatory decisions. Some, such as the Toxic Substances Control Act, require that specific effects of chemical substances, including mutagenicity, be considered in light of the benefits provided by those chemicals in order to ensure that human exposure does not result in an unreasonable risk. This means that the extent of the potential risk must be quantified before decisions are made.

The task of quantifying potential mutagenic risks associated with exposure to chemical mutagens is very complex, and current capabilities require that

many assumptions be made. Extrapolations must be made between species if animal data are used to estimate human genetic risk or between tissues in order to use data from somatic cell biomonitoring to estimate heritable genetic risk. In addition, uncertainties about the exposures that humans receive and extrapolations from effects at high experimental levels to the types of effects expected from much lower environmental levels further compound the problems. Thus, quantitative mutagenicity risk assessments are not scientifically rigorous, because the data base needed to support the extrapolations is not yet complete.

A workshop entitled Approaches for Improving the Assessment of Human Genetic Risk: Human Biomonitoring was held in December 1982, to identify the types of experimental approaches that are required to eliminate some of the assumptions and uncertainties of mutagenicity risk assessment. The approaches identified for using biomonitoring data as a basis for building bridges between experimental mammals and humans are discussed in this paper with the goal of providing direction for the future research that will be required to improve the scientific basis for mutagenicity risk assessment. Emphasis was placed on practical ways to obtain data that are useful in estimating genetic risks. The workshop analyzed available techniques, their usefulness, their limitations, and possible methods for improvement. The impact that increases in the mutation frequency may have on the incidence of human genetic disease was not considered to be in the scope of this workshop. The discussions presented here focus on the workshop proceedings. However, it should be noted that several related publications dealing with the monitoring of humans for detection of genetic damage and/or assessment of genetic risk have appeared since the workshop was held (e.g., ICPEMC 1983 a-f, Streisinger 1983, Ramel 1983, Hook 1983, Miller 1983, Matsunaga 1983, Ashby 1983, Ehrenberg et al. 1983, and Lyon 1983).

II. METHODS FOR BIOMONITORING EXPOSED POPULATIONS

Methods of monitoring human populations for purposes of genetic risk assessment may be classified as direct or indirect, depending on whether extrapolations are necessary for estimating an effect in humans.

A. DIRECT METHOD

The direct method involves the search for mutational effects in human populations exposed to a potential mutagen. It can be used when there is a large population of children of exposed persons available for study. This method was first applied to the study of children whose parents had been exposed to the atomic bombs (Neel and Schull 1956). These children are monitored with respect to a battery of phenotypes, and, when variants are encountered, studies are undertaken to determine whether they are mutations. The observations that might be made on children are of three types: morphological, cytological, and biochemical (Bloom 1980; NAS 1982; Neel 1971, 1981, 1983; Neel and Rothman 1981). The "morphological" observations include the frequency of dominant mutations, congenital defects and stillbirths, altered physical growth and development, and reduced survival. The cytological data include scoring for an array of chromosomal abnormalities. The biochemical approach involves a search for mutant proteins not present in either parent. Because most genetic diseases involve protein alterations, the biochemical approach yields less ambiguous results than the morphological and cytological approaches. However, this method requires a higher level of technology.

Until recently, the biochemical approach in humans and experimental animals has employed one-dimensional electrophoresis and quantitative enzyme level determinations (Neel 1979; Neel et al. 1979; 1980a, 1980b; Satoh et al.

1983). A development of potentially great importance for biochemical monitoring is the advent of two-dimensional polyacrylamide gel electrophoresis (2-D PAGE). This method permits the separation of proteins on the basis of both charge and size on a slab gel; as many as 1000 of the different polypeptides contained in a single cell-type can be detected. Although not all of these can be scored unequivocally for genetic variation (electrophoretic or quantitative), it appears that at least 200 polypeptides potentially suitable for monitoring purposes can be identified from the components of a venous blood sample (Neel et al., in press). Computer algorithms for both the enhancement of these images and their scoring are under development in several laboratories (e.g., Skolnick et al. 1982, Miller et al. 1982, Brown and Ezer 1982).

These developments, if they realize their early promise, could dramatically improve the monitoring of human populations for genetic damage. There are limitations, however. Given the low spontaneous frequency of the mutational events detectable in such gels (3-5 x 10^{-6}/locus/generation), the population size required for an adequate test of an altered mutation rate is massive in cases of low-level exposures. Based on an ability to screen for 200 proteins, the number of observations necessary to test for an increase in the mutation rate of the order of 100% would be at least 10,000 to 12,000 offspring. Such large populations are not likely to exist for the majority of exposure situations that one would like to assess. Because of the requirement for large populations and the fact that most exposures to mutagens will involve low dosages, this method is expensive and will be of limited value scientifically in routine biomonitoring of small groups. However, with appropriate exposures, it may be useful in a coordinated effort pooling offspring from several high risk groups.

Despite the technical difficulty, studies of human germinal mutation rates

are essential in order to understand the increases (if any) in transmitted genetic damage following exposure to mutagens. Such studies are also necessary as part of the basis for extrapolating from animal studies to humans. Thus, it is essential that at least one comprehensive study of a sufficiently large or appropriately pooled "worst case" population be conducted in conjunction with observation on a variety of "presumptive" indicators of mutation. This proposition will be discussed more fully later.

Three pilot studies involving the application of the 2-D PAGE technology either to the evaluation of the effects of a potentially mutagenic exposure or to developing baseline data are being initiated. One involves the children of the atomic bomb survivors of Hiroshima and Nagasaki, who already have been subjected to extensive studies including one-dimensional electrophoresis of blood proteins (Neel et al. 1980b). This pilot study should therefore yield data on the relative efficiency of the two-dimensional method in comparison to the one-dimensional approach. The second pilot study involves children born to Japanese parents who, prior to and during World War II, were engaged in the manufacture of sulphur mustard and other military gases (Neel et al., in press). The third, in Ann Arbor, Michigan, utilizes samples from the placentas of newborn infants and blood samples from their parents.

Because the task of conducting a study using the 2-D PAGE approach to determine human mutation frequencies will be formidable, one may question the wisdom of initiating such studies. To place this in perspective, however, it is necessary to examine an alternative direct method for estimating genetic risk--phenotypic monitoring. The scoring of sentinel phenotypes has been discussed for many years, but the limitations of this approach (i.e., the rarity of individual genetic diseases necessitating the use of large populations)

are recognized to be severe, particularly because the diagnosis for some of the relevant genetic disorders is not always unequivocal. In order to define the mutation frequency in humans, relatively sensitive, direct, and unambiguous approaches must be used. At this time, the 2-D PAGE method seems to be the most feasible approach.

The recent advances in molecular genetics begin to raise the possibility of an approach for estimating mutation rates at the DNA level. A wide battery of restriction-site endonucleases is now available, with each enzyme able to recognize a somewhat different site at which it will cleave DNA. The action of these endonucleases is to cut DNA into fragments of varying lengths. A DNA mutation appearing for the first time in a child would manifest itself as a DNA fragment in an endonuclease digest of that person's DNA which was different in size from any of the fragments obtained from the parents. Although the theory is simple, much experimentation will be necessary to determine which enzymes in combination with which DNA probes yield the most suitable material. A potential problem is translating the implications of any finding with reference to its phenotypic impact on the child. For this reason, it would seem wise to work with defined probes, e.g., probes such as those that exist for the α - and β -chains of hemoglobin or for a number of different enzymes.

B. INDIRECT METHOD(S)

In an indirect approach, tissue samples from exposed persons are analyzed for genotoxic damage, or body fluids are tested for the presence of mutagens. Extrapolations from tissue to tissue are then made in order to predict the risk of genetic disease in future generations. Available methods involve the study of genetic endpoints, including mutations and chromosomal aberrations, or determinations of chemical interactions with DNA.

1. Genetic Endpoints

a) Germ Cells--

For obvious reasons measurements of mutations or other genotoxic effects in germ cells are more appropriate for assessing heritable genetic risk than are similar measurements in somatic tissues. There are at least six indirect tests that can be performed on germinal tissue (i.e., semen samples) from human populations. Four of these tests measure effects on sperm cells, including azoospermy or alterations in sperm motility, morphology, or capacitation (Wyrobek et al. 1983a, b). The remaining approaches utilize other cytological techniques (i.e., analysis of the number of fluorescent Y body chromosomes per sperm [YFF] and hamster egg fertilization with human sperm). Most of the tests for endpoints that may have a genetic basis, such as the putative Y-chromosomal aneuploidy of YFF sperm (Beatty 1977, Kapp et al. 1979) and alterations in sperm morphology (Wyrobek et al. 1983a), are not yet well characterized genetically. Besides this limitation, all of these tests are not applicable to females because of the inability to study ova.

Based on mouse radiation data, the frequency of mutations recovered from females following long-term exposures is considerably lower than that recovered from males exposed in a comparable fashion (Russell 1977). Preliminary data indicate that this is also true for exposures to the chemical mutagen ethylnitrosourea (Russell 1982). It has been proposed that this decreased mutational expression is due to more extensive repair of premutational DNA lesions in the oocyte than in male germ cells. In the absence of conflicting data, the same situation is assumed to exist in humans. Thus, studies restricted to male germ cells may overestimate the true risk for the entire population. Based on these considerations, available human germ cell systems are not yet adequate for predicting genetic risks.

b) Somatic Cells--

There are several genotoxic endpoints that can be measured in somatic tissue (Table I), and the information generated from such measurements can be used to indirectly monitor human populations for heritable genetic damage. Some of the tests measure direct genetic alterations, gene mutations or chromosomal aberrations, while the remainder measure other endpoints indicative of genotoxic damage. Most of these tests can be conducted in both humans and experimental animals, providing a means for correlating epidemiological and clinical data with respect to adverse health outcomes. Because these tests monitor for *in vivo* events, they offer several advantages: (i) they detect genotoxicity from agents whose *in vivo* effects are dependent upon metabolic or pharmacokinetic factors, (ii) they potentially are able to determine the effects of complex mixtures, and (iii) for humans and animals, they may detect heterogeneity for individual susceptibilities to genotoxicants.

(1) Gene mutations--Several somatic cell mutagenicity tests for *in vivo* studies have already been developed, or are in developmental stage. These include: (i) the detection of 6-thioguanine resistant (TG^r) T-lymphocytes arising *in vivo* in humans (Strauss and Albertini 1979, Albertini 1982) or in animals (Recio et al. 1983, Gocke et al. 1983), (ii) the enumeration of mutant hemoglobin-containing mature red blood cells (RBCs) arising *in vivo* in humans or animals (Stamatoyannopoulos and Nute 1983, Bigbee et al. 1983), and (iii) the recognition of surface antigen changes arising *in vivo* on human RBCs (Bigbee et al. 1983). Furthermore, both spontaneous and induced HLA antigen loss mutants can be quantified *in vitro* in human β -lymphoblasts (Pious and Soderland 1977, Kavathas et al. 1980), thus suggesting a method whereby analogous mutants arising *in vivo* in human T-lymphocytes might also be determined.

TABLE I. SOMATIC CELL BIOMONITORING SYSTEMS

End points	Humans		Animal	
	Existing or in Development	Potential	Existing or in Development	Potential
Genetic				
Chromosomal	a. Chromosomal Aberrations	a. Surface antigen loss = chromosome loss (e.g., "Y" antigen) b. Micronucleus test	a. Chromosomal aberrations b. Micronucleus tests	a. Surface antigen loss = chromosome loss (e.g., "Y" antigen)
Specific Locus	a. TG^r lymphocytes b. Mutant hemoglobin containing RBC's c. Glycophorin A surface mutants: MN blood group RBC's	a. Drug resistant lymphocytes diphtheria toxin ouabain b. Surface antigen loss mutant lymphocytes HLA allotypes c. Differentiation antigen loss	a. Mouse spot test b. Granuloma pouch assay c. TG^r lymphocytes d. Mutant hemoglobin containing RBC's e. Lymphocyte surface allotype mutants	a. Drug resistant lymphocytes b. Surface antigen loss Mutant lymphocytes (e.g., H-2)
Indicative of mutagenic effect				
DNA damage/ repair	a. DNA adduct formation Chemical Immunological b. UDS: Spontaneous Induced c. DNA strand breaks d. SCE's	a. Cloned cells DNA technology restriction fragment length polymorphisms	a. DNA adduct formation Chemical Immunological b. UDS: Spontaneous Induced c. DNA strand breaks d. SCE's	a. Cloned cells DNA technology restriction fragment length polymorphisms

Two methods currently are available for detecting TG^r T-lymphocytes arising *in vivo* in humans--i.e., autoradiography (Strauss and Albertini 1979, Albertini 1982) and cloning (Albertini et al. 1982, Albertini 1982, Strauss 1982, Morley et al. 1983). Although each has its advantages, the cloning technique allows recovery and study of the TG^r cells and has allowed a biochemical demonstration of the mutant character of these cells (Albertini et al. 1982, Morley et al. 1983). Lymphocytes usually are obtained for testing by obtaining peripheral blood. However, other sources, such as skin, liver, various tumors, and/or lymph node tissues, can be used. The organ specificity of toxic and/or mutagenic agents potentially can be assessed by quantitating TG^r T-lymphocytes using these techniques because of restricted lymphocyte migration *in vivo* (Gallatin et al. 1983). Some population studies have already been conducted, and "mutagenic" effects have been demonstrated *in vivo* in humans (Strauss and Albertini 1979, Albertini 1982) and in mice (Gocke et al. 1983). Human-animal comparisons are feasible and gene cloning techniques allow for the possibility of comparisons being made at the DNA level.

Many mutant hemoglobins (HbG) have been identified and structurally characterized in humans. Several of these could be useful marker mutants (i.e., hemoglobins S, C, E, Wayne and Cranston). The various mutant hemoglobins result from a variety of structural changes in either the alpha or beta chain of hemoglobin. Identification of these mutant hemoglobins, therefore, is based on the knowledge of their specific changes. Such identification avoids the possible confounding effects of "phenocopies" inherent in assays that detect protein deficiencies. With respect to experimental animals, the ability to test for mutant HbGs is not as developed as it is for humans, although a mutant hemoglobin in the mouse has recently been described (Popp et al. 1983) and schemes for

using interspecies structural differences among HbGs have been suggested (Stamatoyannopoulos and Nute 1983). Different mouse strains have different HbGs, including several with known amino acid differences. Potentially, systems could be constructed with affinity antibodies developed in order to test for mutant HbGs in mice.

For purposes of qauntifying heritable genetic risks, the various tests using somatic tissues are limited. The most obvious restriction is that they are performed with somatic tissue. Thus, tissue to tissue extrapolations must be made in order to make predictions of transmissible genetic risk. Additionally, for risk assessment purposes, mutational events rather than mutant cells are of interest. However, in somatic cell tests, it is mutant frequencies rather than mutational events that are quantified. It will be difficult to quantify the latter because little or nothing is known about _in vivo_ cell generations or cell kinetics (e.g. clonal expansion, _in vivo_ selection, sizes of the populations at risk, cell killing, etc.).

There is also the general difficulty with somatic cell tests in defining the genetic basis of the phenotypic changes at the somatic cell level. Although this difficulty has been overcome for TG^r T-lymphocyte and mutant HbG tests, several potentially useful somatic tests have been abandoned because of the presence of "phenocopies." An early candidate test determined the frequency of antigen-loss variants (ABO antigens) of mature human RBCs (Atwood 1958; Atwood and Scheinberg 1958, 1959). Another test proposed that mutation of the X-chromosomal gene for the enzyme glucose-6-phosphate dehydrogenase (G6PD) could be detected in polymorphonuclear white blood cells (WBCs) by cytochemical methods (Sutton 1972, Sutton 1974). Also, rare variant RBCs containing fetal hemoglobin (HbF) in amounts sufficient to be detected were found in human

peripheral blood (F-cells) (Sutton 1972, Sutton 1974, Stamatoyannopoulos et al. 1975, Wood et al. 1975). It was postulated that these F-cells arose because of a mutation in the structural gene for the beta chain of normal hemoglobin, an event which occurred in rare erythroid precursor cells. Therefore, F-cells were proposed as indicators of somatic cell mutation occurring in vivo. All three endpoints have been rejected as indicators of somatic cell mutations because non-genetic factors such as physiological or pathological conditions, or changes in assay methods could result in cells with the indicated phenotypes (Atwood and Petter 1961, Stamatoyannopoulos 1979). In none of these systems could "phenocopies" be differentiated from true mutant cells.

(2) Chromosomal aberrations--It is possible to study cells in various tissues for chromosomal aberrations and micronuclei.

(a) Chromosome breakage and rearrangements--Several approaches are available for studying the frequency of chromosomal aberrations in peripheral blood lymphocytes, bone marrow cells, and germ cells (Preston et al. 1981). Such cytogenetic studies allow comparisons between effects in somatic cells and effects in germ cells, as well as comparisons between species. Chromosomal aberrations provide unequivocal evidence of genetic damage and thus constitute a relevant endpoint for reproductive hazards. Furthermore, many carcinogens have been shown to be clastogens. Generally accepted principles for the conduct of tests and the scoring of results have been developed (Preston et al. 1981). Considerable research has been conducted to assess spontaneous frequencies, the clastogenic effect of physical and chemical agents, and to define the technical variables in the techniques. The limitations of cytogenetic analysis are that it is labor intensive and requires a high level of experience for accurate scoring. In addition, the data base on interindividual variation,

persistence of lesions, and the sensitivity of peripheral blood lymphocytes to various classes of chemicals is relatively small.

(b) Micronucleus test--Micronuclei are small membrane-bound nuclear fragments that arise from acentric chromosomal fragments or from entire chromosomes which are excluded from within one of the daughter nuclei during cell division (Heddle et al. 1983). Lagging fragments result from clastogenic damage while lagging chromosomes result from a disturbance of the mitotic apparatus. Micronuclei can be scored in any proliferating cell population, but the most common procedure involves the scoring of micronuclei in the polychromatic erythrocytes (PCEs) in mammalian bone marrow (Schmid 1976, Heddle et al. 1983). However, because of the short lifespan of PCEs (approximately 24 hr) and the requirement for bone marrow samples, this approach is largely restricted to tests that use acute exposure regimes in experimental mammals. Although rbcs with micronuclei can be detected in the peripheral blood of mice, the ability of the spleen to remove such rbcs in most other mammals, including humans, precludes the use of this approach for studies of environmental effects. Attempts to study micronuclei in other cell populations, such as buccal smears (Stich et al. 1982) or biopsy material from endoscopic examinations (Heddle et al. 1983), have been conducted with only partial success. The usefulness of micronucleus tests for detecting somatic genotoxic damage, therefore, remains in question.

Although it is possible to perform somatic cell biomonitoring tests on several different cell-types, the difficulty of obtaining most tissues necessitates that peripheral blood will be the main source of tissue for human biomonitoring studies. Blood tissue is readily available, and thus somatic cell systems offer the key advantage of permitting the collection of data from small populations of individuals. However, measurements of mutational events

in somatic cells also have deficiencies. Among these are the limited data base for chemicals, the insensitivity of some of the endpoints as indicators of genotoxic damage, the lack of appropriate bridging models to predict heritable effects, and the lack of evidence for a correlation between elevated levels of mutations in somatic cells and an increased risk for adverse health effects.

2. Measurements of DNA Damage

Various endpoints that potentially indicate mutagenesis can be detected in both somatic and germinal tissue. These include sister chromatid exchanges (SCEs), chemical interactions with DNA, and DNA repair. Of these three approaches, the detection of SCEs is at the most advanced stage of development. Difficulties are encountered in all of these methods, however, because of varying replication rates and repair capabilities in different cell-types and because of the restriction of germ cell measurements to males. Furthermore, positive findings with these tests cannot be equated with an increase in the frequency of mutations. Nonetheless, the measurement of SCEs in peripheral lymphocytes is a relatively easy and sensitive test, and several sensitive techniques are being developed for measuring DNA damage. These approaches may be used to provide information on internal dosages resulting from human exposures to chemical substances, and as such may be employed to provide a common denominator for tying human biomonitoring and animal testing methods together.

a) Sister Chromatid Exchanges--

SCEs are sensitive indicators of certain types of DNA damage (Latt et al. 1981). SCE tests are easy to conduct in vitro, and most mutagens induce SCEs. In vivo tests are also possible, allowing interorgan and interspecies comparisons to be made. The most useful approach for interspecies comparisons involves combined in vivo - in vitro tests, where exposure to the mutagen occurs in

vivo, but bromodeoxyuridine (BrdUrd) treatment and cell replication occur in vitro. Certain classes of agents are more effective inducers of SCEs than others. Agents that cause DNA base damage (e.g., ultraviolet light and alkylating agents) are good inducers of SCEs, while agents that primarily break the DNA backbone (e.g., X-rays and bleomycin) are poor inducers of SCEs. Variability in test parameters and a lack of understanding about the mechanism of SCE formation limit the use of the technique in risk assessment. Variability in the measurement of SCEs must also be considered. Biological variability may be associated with sex, age, genotype, or diet, and technical variability may be associated with various culture conditions (e.g., the amount of BrdUrd used, the source and type of serum, temperature).

b) DNA Repair--

The repair of DNA damage involves a sequence of biochemical steps that can be studied by techniques that detect the formation and removal of damaged bases, the insertion of new bases during repair synthesis, or the formation and sealing of DNA breaks during excision. Few of these techniques are amenable to rapid, simple, and inexpensive use in screening large numbers of individuals. Peripheral lymphocytes, which are the most available cell type in population studies, are not ideal for the measurement of repair; their repair capacity is depressed in comparison to many other somatic cell types, probably because they have low levels of DNA polymerase alpha (Scudiero et al. 1976).

Available techniques for measuring DNA damage/repair include: (i) alkaline sucrose gradients for single strand breaks; (ii) tritiated thymidine incorporation, isopycnic gradients, and BrdUrd photolysis for repair replication; (iii) direct measurement of loss of adducts with labeled mutagens; (iv) and analysis of DNA monomers by high pressure liquid chromatography (HPLC). With the exception of

tritiated thymidine incorporation to measure repair replication, these methods are time-consuming, need specialized skills, and can be expensive (Cleaver 1974). Some of the methods, such as direct measurement of the loss of radiolabeled adducts, are not suitable for use in humans. Alkaline elution has potential (Petzold and Swenberg 1978) but is subject to complex artifacts, and interpretations of the resulting data are rarely simple.

The most readily applicable method for assessing DNA repair in screening studies is the measurement of the patching step of repair. Measurements can only be made by autoradiography or by scintillation counts of the incorporation of ^{3}H-thymidine (^{3}H-TdR) into the DNA of cells in which semiconservative DNA replication is negligible (Cleaver 1974). A drawback to this technique is that ^{3}H-TdR uptake may differ between individuals, not only because of DNA repair differences but also because of differences in thymidine kinase, phosphorylases, or pyrimidine nucleotide pools (Cleaver 1967). Autoradiography is more time-consuming but does allow cell by cell comparisons of unscheduled DNA synthesis (UDS). The most rapid method for measuring UDS is scintillation counting of cells exposed to ^{3}H-TdR in the presence of inhibitors of DNA replication (e.g., hydroxyurea). However, scintillation counting only registers a net change in counts without indicating the amount in each cell. Residual replicative DNA synthesis can therefore confuse the results obtained by scintillation counts, but not those obtained by autoradiography. Furthermore, high concentrations of hydroxyurea can also interfere with repair synthesis, further confusing interpretation of the results. Additionally, levels of UDS can vary greatly between cell types and differences in UDS between individuals may not reflect only differences in DNA, but also differences in levels of enzymes responsible for the incorporation of exogenous TdR. Finally, although many mutagens elicit

an increase in UDS, some known promutagenic lesions, such as O^6-methylguanine do not. The failure to detect the repair of such lesions further complicates extrapolation of UDS data to human health risks.

The use of DNA repair to detect genotoxic damage should be viewed with reservations. DNA repair primarily occurs during the initial 12-24 hours after exposure making a delayed assessment of repairable damage extremely difficult. Screening for DNA repair also presents a conceptual problem, because the techniques do not measure damage resulting in mutagenesis per se. Rather, they measure the cell's attempts to correct such damage. The correspondence between repair and genetic damage is complex and depends on parameters such as the cell cycle and type and extent of damage induced. In replicating cells there is, in a sense, competition between replication and repair. Mutations may result even if error-free repair occurs, if the damaged DNA replicates first. In addition, if the type or extent of DNA damage is such that it is not repaired and persists, it can cause disturbances in DNA replication and produce sister chromatid exchanges or chromosomal aberrations, mutations, carcinogenic transformation, etc. On the other hand, certain kinds of DNA alterations that lead to mutation and/or cancer either do not stimulate repair processes at all, or do so to such a small extent that detection is not practicable. Thus, difficulties exist in using methods for detecting DNA repair to predict genetic risk.

Mutagenic chemicals, such as the intercalating agents ethidium bromide, acriflavine, actinomycin D, and adriamycin, do not invoke DNA repair synthesis even at high doses (Cleaver 1968, Painter 1978, Painter and Howard 1978). Similarly, X rays are mutagenic and carcinogenic but induce very little repair synthesis (Painter and Young 1972, Regan and Setlow 1974). Also, some metals that are suspected carcinogens do not elicit detectable repair (Painter 1979).

Thus, while detection of repair synthesis is useful after exposure to some mutagens or carcinogens, resolution of important differences within a population that are relevant to mutagen/carcinogen damage will not always be possible by this method.

c) Measurement of Chemical Binding to DNA--

The major, current methods for measuring DNA lesions are radioactivity, immunoassays, and fluorescence. Each of these techniques has certain strengths, but also some major weaknesses that limit their usefulness.

(1) Radiolabeled DNA probes or mutagens--DNA can be uniformly radiolabeled by feeding cells radioactive precursors to yield DNA probes which can then be reacted with modifying agents. These techniques are not applicable for the detection of DNA lesions in human specimens from environmental exposure. It is also unfortunate from a practical standpoint that radiolabeled substances, particularly those of high specific activity needed for ultratrace work, present hazard, inconvenience, cost, and disposal problems. Post-labeling techniques with radiolabeled probes can be used to detect DNA lesions in human specimens (Franklin and Haseltine 1983, Randerath et al. 1981) with high sensitivity and fingerprinting capability (Gupta et al. 1982), but the same practical limitations remain because they require the use of radioisotopes.

(2) Immunoassays--Radioimmunoassay and related procedures are important techniques for ultratrace analysis of DNA lesions. For example, Hsu et al. (1981) detected 3 fmol (3×10^{-15} mole) of a benzo(a)pyrene-DNA adduct with an immunoassay. Poirier et al. (1979) similarly detected various acetylaminofluorene-DNA adducts with detection limits, depending on the immuno-substrate, from 0.5 to 160 pmol, and achieved a detection of 1-2 fmol of platinum DNA adducts per 50 ug of DNA (Poirier et al. 1982, Poirier 1983). Because immunoassays are indirect

methods, there is a potential for inaccuracy (Houck et al. 1980, Julliard et al. 1980). Another limitation is that a different immunoassay is needed for each DNA modification.

(3) Fluorescence--Detection of complexes between mutagens and DNA by fluorescence has recently been reviewed (Vigny and Dusquesne 1979). The major limitations of this approach are that different structures can have similar flourescent spectra. In addition, some structures are not very fluorescent, and fluorescent contamination can confuse the results.

(4) Measuring damage in DNA by gas chromatography-mass spectrometry--McCloskey and co-workers used gas chromatography (GC) with fused silica columns for the analysis of nucleic acid bases (Gelijkens et al. 1981). The bases were converted into N,O-peralkyl (methyl or ethyl)N-trifluoroacetyl derivatives, and, after GC separation, were detected by either flame ionization detection, nitrogen-phosphorous detection, or electron impact mass spectrometry. These solutes showed good GC behavior, including a good sensitivity (low pg level, 10^{-10}g). There is a potential for higher sensitivity by gas chromatography with electron capture detection (GC-ECD) and gas chromatography with negative ion chemical ionization detection (GC-NICI-MS). Detection limits at the femtogram level for the analysis of standards of cytosine and 5-methylcytosine have recently been established (Nazareth et al., in preparation; Gemal et al., in preparation).

The methodology being developed to measure damage to DNA will consist of the following steps: (1) purification of the DNA; (2) enzymatic or acid hydrolysis of the DNA, releasing monomers; (3) separation of damaged monomers from normal monomers by HPLC; and (4) chemical labeling of the damaged monomers with "direct electrophores," followed by characterization and quantification of these

electrophore-labeled monomers by GC-ECD or GC-NICI-MS.

Preliminary results with GC-ECD and GC-NICI-MS methodology suggest, at least for certain standards, that damaged DNA bases can potentially be quantified at the 10^{-16} mole level. This translates into an ability to measure nearly 100 damaged monomers per human cell given a sample of 2×10^6 cells or 10 ug of DNA (a typical number of cells or amount of DNA from a typical human microbiopsy). This amount of DNA corresponds to that obtainable from about two Petri dishes of cultured cells. However, this technology has not yet been applied to the analyses of actual samples.

Much remains to be learned about specific DNA lesions, their repair, and their implications for mutagenesis and carcinogenesis. Despite the complexities, simple models can prove valuable in making the necessary first steps to improve genetic risk assessment. For initial studies with experimental animals, total DNA alkylation may serve as useful indicators of dose. Eventually, the specific type of alkylation product, rate of specific adduct repair, amount of cell replication, and the probability of mispairing of specific adducts needs to be considered. Although we are presently incapable of accomplishing this goal, the methodology for conducting such studies is developing rapidly.

III. BIOMONITORING ASSAYS AVAILABLE FOR MAMMALIAN EXPERIMENTATION

Both the direct and indirect approaches can be used to estimate the frequency of heritable mutations associated with mutagen exposures in experimental mammals. This is required for the bridge building approaches described later. In animal studies, exposures can be carefully identified and controlled, and cause and effect relationships can be demonstrated. Application of the indirect methods for

both human and animal studies has been described previously. Direct and indirect methods applicable only for animal experimentation will be described in this section. These methods are useful for defining intertissue relationships and for making comparisons with human data in order to strengthen the basis for extrapolating between species.

Whole mouse tests for putative heritable gene mutations are generally considered the most valid experimental approaches for making quantitative mutagenicity risk assessments (NAS 1982, Lyon 1983). Among these are the morphological and biochemical specific locus tests and tests for dominant mutations causing skeletal defects or cataracts. Other available tests score for chromosomal aberrations; these tests include the heritable translocation test, dominant lethal test, and X chromosome loss test. All of these tests, except perhaps the dominant lethal test, which cannot be shown to respond only to mutagenic events, may be used for quantifying genetic risk. An indirect estimation of heritable genetic effects in mice can be performed using the mouse spot test.

A. DIRECT METHOD

1. Gene Mutations

a) Specific Locus Test--

The morphological specific locus test has several advantages that arise from its long historical use in genetic toxicology and genetic risk assessment (Russell et al. 1981a). For this test, there are good historical control data with reliable spontaneous mutation frequencies. However, much of the available information comes from radiation studies (Russell 1951). The data base on chemicals evaluated in the specific locus test in intact mammals is small. Only 25-30 chemicals have been evaluated, and of these, there are

adequate data for only about 15; of the 15, roughly half are inconclusive. There are dose-response data in specific locus tests on only four chemicals: procarbazine, mitomycin C, triethylenemelamine, and ethylnitrosourea. It is difficult to decide on the meaning of negative results in such tests, because large numbers of animals are required to preclude modest (two- to fourfold) increases in mutation frequencies. Nevertheless, the specific locus test has the advantage that the genetic events are reasonably well defined, and it does seem to detect both gene mutations and deletions.

In the morphological specific locus test, mutations are detected at only seven loci, and the number of loci is not readily expandable. The ability to detect genetic events at more loci is one of the advantages of the electrophoretic specific locus test (Valcovic and Malling 1973; Johnson et al. 1981). In this test, there are currently 10 loci at which the two tester strains of mice differ, permitting the detection of both null mutants and electromorphs. There are at least 10 additional loci that can be screened for electromorphs only with the possibility of further expansion. Another advantage of the electrophoretic specific locus test is that it is directly comparable to the electrophoretic monitoring system for human populations that has been developed by Neel et al. (1979) and described above. A real limitation at this stage in the development of the electrophoretic specific locus test is that the data base on chemically-induced mutations is small. No spontaneous mutants have yet been recovered in about 300,000 locus tests, which would suggest a mutation frequency less than or equal to that of the morphological specific locus test. The system described here uses the C57BL/6 and DBA/2 inbred mouse strains; other inbred strains could be used but the number of polymorphic loci would be reduced.

b) Tests for Dominant Mutations--

Tests for dominant mutations that cause skeletal defects (Ehling 1966, Selby and Lee 1981) or cataracts (Kratochvilova 1981; Kratochvilova and Ehling 1979) have been proposed for use in genetic risk assessment. It has been argued that these tests better approximate the dominant human disease syndromes that might be expected in the first few generations after significant mutagenic exposure. The argument has also been made, however, that this advantage may be overstated for the dominant skeletal test (Lovell et al., submitted) and may even be trivial. There may be a serious disadvantage in detecting dominant mutations in particular strains of inbred experimental animals and extrapolating the result to outbred populations; polygenic traits can appear as simple dominants in a particular cross that produces a genotype that is near the threshold of expression. Another factor to consider is that the data base on chemicals is extremely limited for these tests. So far, there are data only for ethylnitrosourea. Relative to testing for skeletal mutations, the test for cataracts offers the advantage that the detection is noninvasive, thereby permitting follow-up breeding experiments to verify the genetic nature of the alteration.

2. Chromosomal Aberrations

a) Heritable Translocation--

The heritable translocation test detects symmetrical reciprocal exchanges of chromosomal material between nonhomologous chromosomes which are transmitted from parents to offspring (Generoso et al. 1980). Translocation heterozygotes are viable and are detected by st ility or semi-sterility. Confirmation is made by chromosome analysis. Because heritable translocations are by definition scored in live progeny, they provide a very definitive and unequivocal measure of clastogenic effects in the germ cells. Consequently, they are

generally considered to be the most important clastogenic endpoint with respect to genetic risk assessment.

The spontaneous occurrence of chromosome breakage-related anomalies in humans is estimated to be 2,400 per million live births (35), many of which result from translocations. For example, approximately 20% of the trisomy 13 disorders (Patau's syndrome) are caused by translocations (Magenis et al. 1968, Taylor et al. 1970), which usually involve the transfer of material from chromosome 13 to chromosome 14. The phenotypic manifestations of translocations in human carriers are varied, ranging from no perceptible effect to mentally-related handicaps (UNSCEAR 1977). The exchange usually involves the transfer of material from chromosome 13 to chromosome 14. Although the total contribution that heritable translocations make toward all chromosomal disorders has not been determined adequately, it may be assumed for purposes of estimating human risk that all newly-occurring translocations are deleterious, if not directly to the carriers, then at least to some of their conceptuses.

b) Sex Chromosomal Abnormalities--

Approximately 0.6% of human newborns have congenital anomalies which result from chromosomal aberrations. These may be unbalanced segregation products of translocations or numerical aberrations (BEIR 1980). Numerical anomalies are known for both autosomes and sex chromosomes.

A numerical sex chromosome-loss test using mice has been described by Russell (1976, 1979a, b). Potentially mutagenic agents can be tested in either sex, and numerical chromosomal disorders can be scored in the F_1 generation. As is the case with most other whole mammal tests, large numbers of animals are necessary to obtain dose-response relationships at low exposure levels, necessitating the use of relatively high experimental doses. If experimental

data on an environmental agent are known, then an estimate of human risk can be attempted by extrapolation. For example, if mouse experiments show that a certain exposure to a chemical agent doubles the incidence of sex chromosome loss (XO), then the incidence of Turner's syndrome in humans may be multiplied by the same factor following "equivalent" exposure.

One has to assume that an agent causing a positive response in a numerical sex chromosome study would also cause numerical anomalies (monosomy or trisomy) among autosomes. Therefore, humans exposed to the chemical agent would be at risk to other diseases caused by autosomal numerical aberrations. Only four chemicals (triethylenemelamine, methyl methanesulfonate, isopropyl methanesulfonate, and hycanthone) and ionizing radiation have been tested in the mouse numerical-sex chromosome anomaly system (Russell 1976). All of these agents are also known to cause dominant lethals. The sex chromosome loss test is the only available test that assesses the capacity of an agent to cause germ cell aneuploidy *in vivo* which results in recognizable offspring.

c) Dominant-Lethal Test--

Dominant-lethal mutations are genetic changes in parental germ cells that cause death of first-generation embryos. It is generally believed that induced dominant-lethal mutations are due to chromosome breakage events (Bateman and Epstein 1971; Generoso, in press). Because most point mutations will not be detected, the dominant-lethal test can only be used to assess an agent's mutagenic potential in qualitative terms. In general, there appears to be a close correlation between the induction of dominant-lethal mutations and the induction of heritable translocations. There are examples, however, of chemicals that effectively induce dominant lethal mutations with very few or no heritable translocations (Generoso et al. 1979). A definitive positive

response in a dominant lethal test serves as a strong indication that a chemical causes genetic damage in male germ cells. However, the dominant lethal test is not a sensitive assay and negative responses are not conclusive.

B. INDIRECT METHOD (MOUSE SPOT TEST)

In the mouse spot test, somatic cell mutations occurring in melanocytes during embryonic development are detected after birth as patches or spots of altered fur color. It is thought that this technique is capable of detecting gene mutations, large and small chromosomal deficiencies, nondisjunction or other chromosome loss, and somatic recombination (Russell 1978, 1979a; Russell and Major 1957). Pregnant mice are treated about 10 days after conception at a time when there are an estimated 150-200 melanocyte precursor cells/embryo at risk. Depending on the crosses used, the fetuses are heterozygous at three or four coat color loci. At birth, offspring are checked for externally visible morphological features and examined for spots 12-14 days later. The animals are then re-checked at least once at 4-5 weeks of age before they are discarded. Data are recorded as the percentage of animals with marker spots. Positive responses in this test demonstrate the test agent is a mammalian mutagen, but because somatic cell events are scored, the data cannot be used to estimate heritable genetic risk directly. Thirty substances have been tested in this manner. Three were solvents used in testing the other chemicals. Of the remaining 27 chemicals, 16 were positive, 6 were negative, and 5 were inconclusive (Russell et al. 1981b). Historical control values vary significantly depending on which solvent was used to administer the test agent. Thus, it is important to compare experimental results with appropriate controls. It is recommended that a

concurrent solvent control of at least 150 animals be used (Russell et al. 1981b). Appropriate animal facilities and properly trained personnel are needed to conduct this test. The test is subject to false positive results. For instance, certain coat color spots resulting from chemical treatment do not have a genetic basis but appear to be due to cell-killing. Errors of differentiation that are not due to genetic damage are also detected in the test.

C. LIMITATIONS OF ANIMAL TEST DATA FOR ESTIMATING HUMAN GENETIC EFFECTS

The close biological and evolutionary relationship between humans and other mammals is the basis for estimating heritable human genetic risk from mouse and rat data. However, there are several limitations associated with using studies in animals for predicting human responses. One is the difficulty of accounting for differences in metabolism, repair, and cell cycle kinetics. Another is the need to extrapolate from high acute dosages, ofter involving long sterile periods, to dosage levels that would be more typical of human exposures. Another limitation in essentially all assays for mutagenesis in germ cells is the shortage of information on females; the great majority of the available information comes from males. Consequently, many assumptions must be made in attempting to project human genetic risk. This leads to the inescapable conclusion that there is no substitute for genetic data from humans to calibrate the experimental systems for risk assessment purposes.

IV. IDENTIFICATION OF HUMAN POPULATIONS EXPOSED TO CHEMICAL MUTAGENS

Considerable effort will be required to collect human data for assessing genetic risk for just one chemical substance will be great, and all sources of

information ought to be drawn upon to select the appropriate human population for study. A number of populations at greatest risk should be identified for potential studies. One is the children of cancer chemotherapy patients. For 1983 it was estimated that there would be in excess of 160,000 cancers that may have long remissions/cures following effective chemotherapy (Table II) (Silverberg and Lubera 1983). Around 29,000 of these cancer patients will be treated at large cancer centers, most with defined protocols of drug administration. A large number of treated individuals will be of child-bearing age. It is reasonable to expect similarly large populations in the years to come. Even if a small fraction of such individuals were employed in an epidemiological study, it is likely that a meaningful cohort could be assembled in a short time. This population is clearly not typical of a normal healthy population, but it provides a model situation where persistent damage to DNA in humans could be investigated and related to the incidence of mutations in their offspring. There are other analogous populations as well, and careful thought should be given in the design of a program to identify and select the most appropriate ones. Information from these studies would be useful for defining the extent of genetic hazard and in validating the animal models as predictors of human risk.

V. DISCUSSION OF APPROACHES FOR THE IMPROVEMENT OF MUTAGENICITY RISK ASSESSMENT

There are many available tests for identifying chemical mutagens. Data from combinations of tests provide a basis for making qualitative assessments of the ability of chemical substances to cause gene mutations, chromosomal aberrations, and other effects that are indicative of interaction with DNA.

TABLE II. ESTIMATES OF INCIDENCES OF CANCERS WITH LONG REMISSIONS/CURES

Tumor	Incidence
Breast Cancers	114,000
Ovarian Cancers	18,200
Hodgkin's Lymphomas	7,100
Non-Hodgkin's Lymphomas	23,600
	162,900

However, only a few tests (i.e., heritable gene mutation and heritable translocation tests in mice) can be used by themselves for quantitatively assessing genetic risk. They are not routine tests, however, and they cannot be used to estimate human genetic risk directly. By default, assessment of genetic risk must be done qualitatively for most chemical substances.

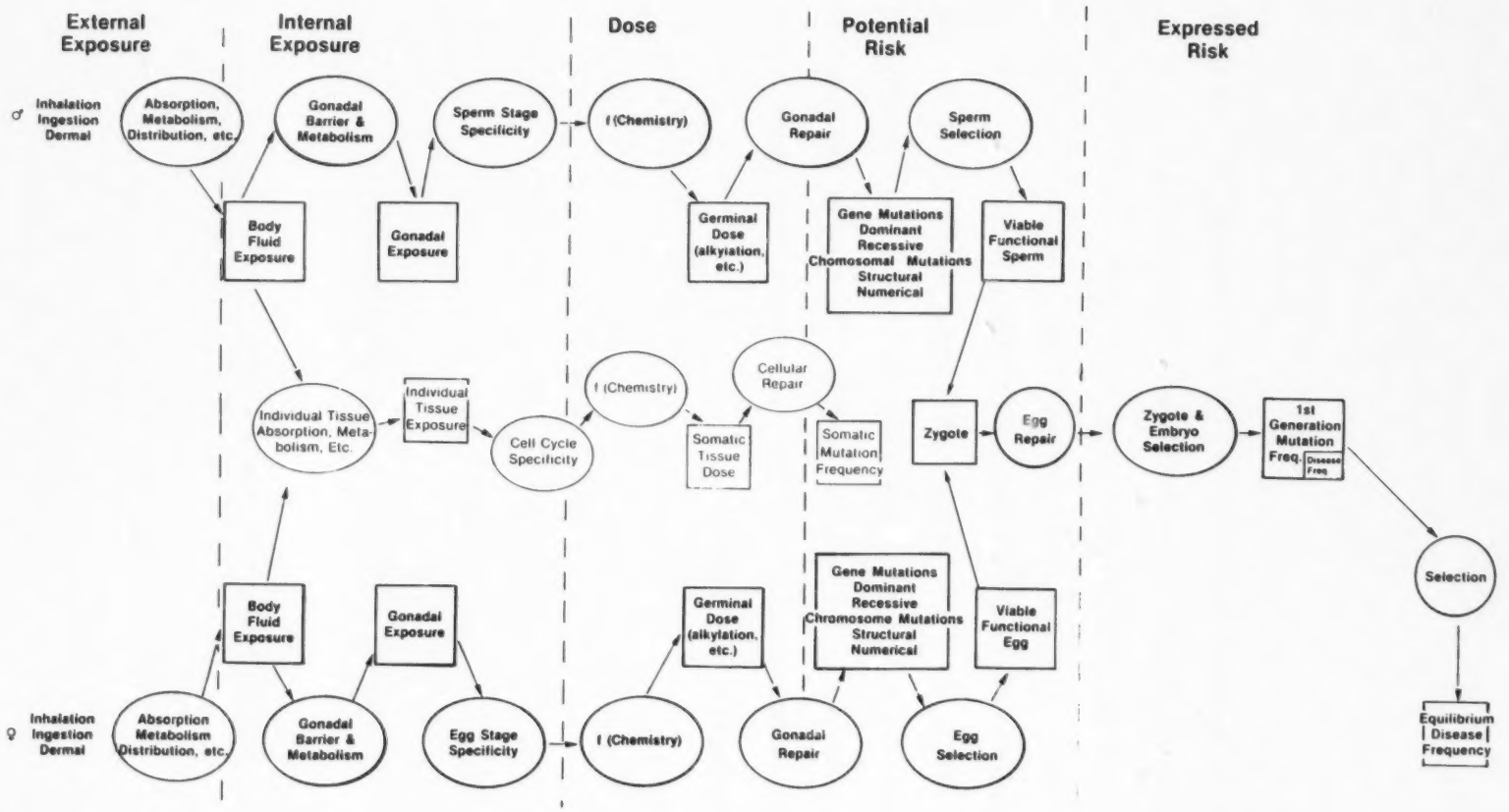
For decision-making by the Federal government, it is no longer adequate to merely qualitatively assess genetic risk; quantitative assessments are needed to balance the risk associated with exposure to a chemical against the benefit of its use. Because our understanding of interorgan relationships is inadequate, human monitoring data cannot be used for such purposes. Many assumptions have to be made before genetic risk can be estimated quantitatively, and the assessments are not scientifically rigorous. Because of practical and legal considerations, it is important to make optimal use of all sources of information in future genetic risk assessment efforts and to develop the science to a point where rigorous assessments can be made.

A. BRIDGING HUMAN BIOMONITORING ENDPOINTS WITH ANIMAL EXPERIMENTATION (Defining the Relationships)

Each test system has advantages and limitations for assessing genetic risk to humans. It is only by determining how the endpoints measured in these tests relate to events that occur in humans that full advantage can be taken of each. Comparative experimentation involving different endpoints, test systems, and chemicals is required to build "intellectual bridges" between the systems.

Studies in mice can be used by themselves to predict mutagenic effects in humans because the same range of steps between external exposure and production of mutant offspring occurs in all mammals (Jackson, in preparation) (Figure 1).

Figure 1. An approach for examining the process leading from external exposure to expression of genetic disease and somatic cell effects is outlined in this Figure. It is derived into five major steps (i.e., external exposure, internal exposure, dose, potential risk, and expressed risk) each represented by a separate column. The top row represents events occurring in the germinal tissue of males, while the bottom row represents the tissue of those occurring in females. The middle row corresponds to somatic cell events. The squares represent potentially measureable events. The circles represent processes that cannot be measured but that can be estimated from measurements of the events represented by squares.


External Exposure
Internal Exposure
Dose
Potential Risk
Expressed Risk
♂
Inhalation
Ingestion
Dermal
Absorption, Metabolism, Distribution, etc.
Gonadal Barrier & Metabolism
Sperm Stage Specificity
f (Chemistry)
Gonadal Repair
Sperm Selection
Body Fluid Exposure
Gonadal Exposure
Germinal Dose (alkyation, etc.)
Gene Mutations Dominant Recessive Chomosomal Mutations Structural Numerical
Viable Functional Sperm
Individual Tissue Absorption, Metabolism, Etc.
Individual Tissue Exposure
Cell Cycle Specificity
f (Chemistry)
Cellular Repair
Somatic Tissue Dose
Somatic Mutation Frequency
Zygote
Egg Repair
Zygote & Embryo Selection
1st Generation Mutation Freq.
Disease Freq.
Selection
Body Fluid Exposure
Gonadal Exposure
Germinal Dose (alkylation, etc.)
Gene Mutations Dominant Recessive Chromosome Mutations Structural Numerical
Viable Functional Egg
♀
Inhalation
Ingestion
Dermal
Absorption Metabolism Distribution, etc.
Gonadal Barrier & Metabolism
Egg Stage Specificity
f (Chemistry)
Gonadal Repair
Egg Selection
Equilibrium Disease Frequency

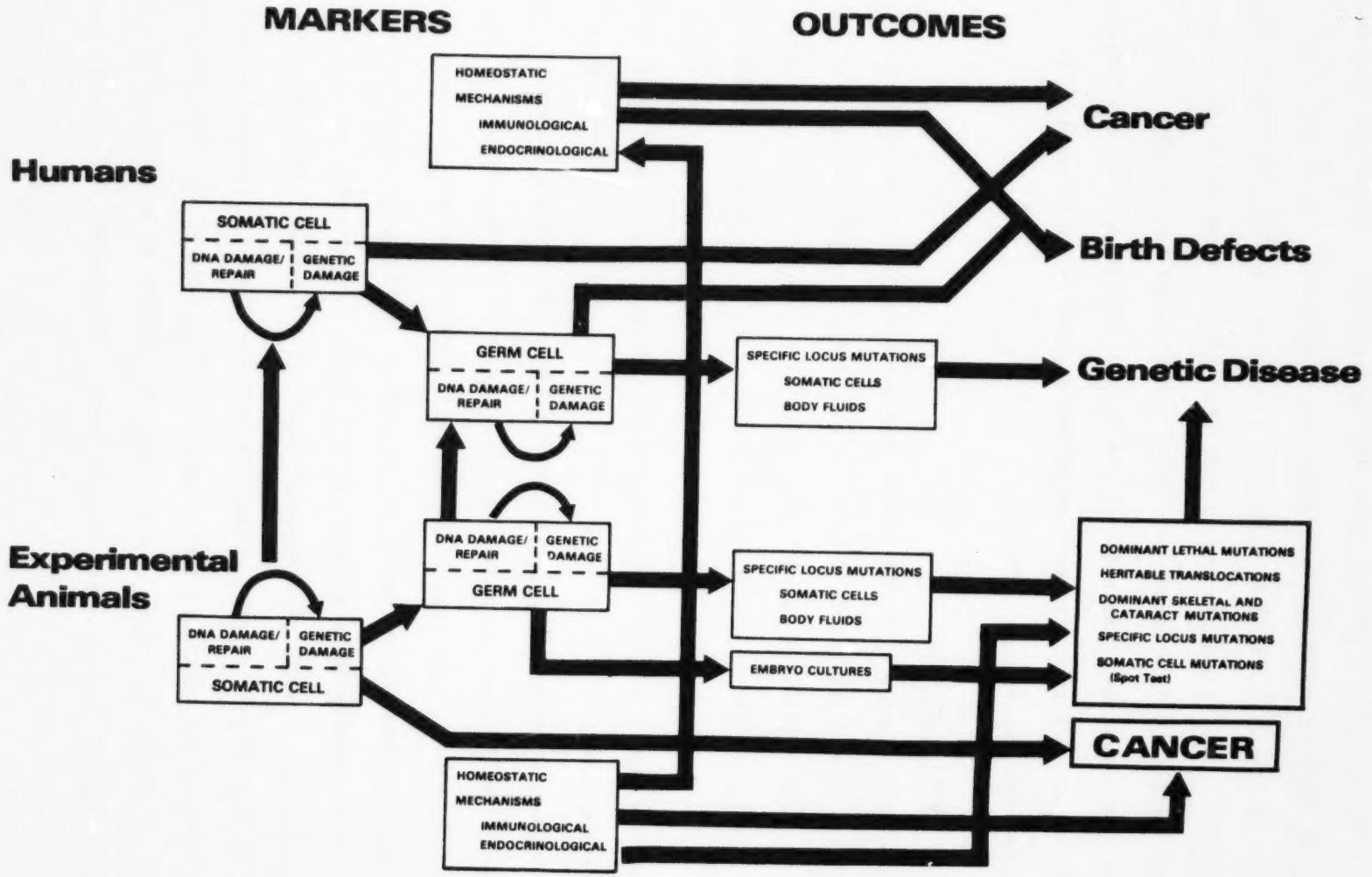
Measurements of somatic cell and germ cell events should be performed in mice in such a way that the relationships between biomonitoring markers and health outcomes of concern (i.e., genetic disease, cancer, and birth defects) can be determined (Figure 2). Such an approach will provide a basis for using biomonitoring endpoints to predict adverse health effects. It will also enable comparisons to be made between different health outcomes in order to determine their sensitivity of expression and predictability.

It is necessary to conduct at least one study in such a manner so that the data generated in an experimental animal study can be compared to the results obtained from similarly exposed humans. Until this is done, the applicability of animal data for human risk assessment will be unknown. A large number of tissues are available for routine study in animal tests, but only a limited variety and amount of tissue will be available for study in "routine" human biomonitoring studies (i.e., blood and sperm). However, for "bridge-building" purposes a larger variety and amount of tissue is potentially available (e.g., tissue that normally would be discarded after surgery on cancer patients).

B. OTHER TYPES OF TESTING NEEDED

Because it has not been nor will it likely be possible to study genetic damage in more than a very few human populations, it will be necessary to rely heavily on animal experimentation and short-term biomonitoring tests to predict human risk. This requires bridge building between human heritable mutagenicity data, other human biomonitoring data, and animal heritable mutagenicity and biomonitoring data. Although the specific models that can be used to make bridges remain controversial, there seems to be some agreement that ratios (or parallelograms as they are sometimes called) involving dosimetry should be explored further (Sobels 1981 a, b; Malling 1981; Lyon 1983). As such

Figure 2. Relationships between biomonitoring endpoints (markers) and disease outcomes of concern. Understanding these relationships is required to be able to use biomonitoring data for estimating risk. This requires comparative experimentation measuring different markers and outcomes in the same or similarly exposed individuals. This diagram shows a comparative approach for defining interorgan and interspecies relationships.


MARKERS
OUTCOMES
HOMEOSTATIC
MECHANISMS
IMMUNOLOGICAL
ENDOCRINOLOGICAL
Cancer
Humans
SOMATIC CELL
DNA DAMAGE/ REPAIR
GENETIC DAMAGE
Birth Defects
GERM CELL
DNA DAMAGE/ REPAIR
GENETIC DAMAGE
SPECIFIC LOCUS MUTATIONS
SOMATIC CELLS
BODY FLUIDS
Genetic Disease
DNA DAMAGE/ REPAIR
GENETIC DAMAGE
GERM CELL
SPECIFIC LOCUS MUTATIONS
SOMATIC CELLS
BODY FLUIDS
DOMINANT LETHAL MUTATIONS
HERITABLE TRANSLOCATIONS
DOMINANT SKELETAL AND CATARACT MUTATIONS
SPECIFIC LOCUS MUTATIONS
SOMATIC CELL MUTATIONS (Spot Test)
Experimental Animals
DNA DAMAGE/ REPAIR
GENETIC DAMAGE
SOMATIC CELL
EMBRYO CULTURES
CANCER
HOMEOSTATIC
MECHANISMS
IMMUNOLOGICAL
ENDOCRINOLOGICAL

studies are conducted, knowledge of biological processes rather than statistical models can start to drive the risk assessment procedure. Flexibility should be maintained so that the risk assessment process works differently when different amounts of data are available and so that the level of sophistication can be increased as better data become available. In addition to providing more precise risk assessments, maintaining the flexibility needed to better risk assessments would foster the application and support of basic research for the risk assessment procedure.

1. Cell-Specific Effects

The effects caused by a chemical in one cell-type might not predict its effects in a different cell-type. Thus, it is important to consider cellular specificities of certain chemicals. For example, based on studies investigating carcinogen metabolism, it is known that the metabolic competence of different cell populations within the target organ can affect the extent of DNA adduct formation as well as toxicity-induced compensatory cell proliferation. For example, removal of covalently bound 2-AAF has been shown to be similar in hepatocytes and nonparenchymal cells of the liver (NPC), whereas major differences have been demonstrated for removal of O^6-alkylguanine (Swenberg et al. 1984). In the latter case, cell specificity is also dependent on chemical reactivity with DNA. The major premutagenic lesion induced in DNA by SN_1 methylating agents is O^6-methylguanine (O^6 MG) (Singer 1975). In contrast, ethylating agents produce a greater proportion of O-alkylated premutagenic pyrimidines, relative to O-alkylation of guanine. These differences can perhaps be best appreciated if one considers some recent data (Swenberg et al. 1984) which has shown that O^4-ethylthymidine progressively accumulates in hepatocytes over 4 weeks of exposure to diethylnitrosamine (DEN). In contrast, O^6-ethylguanine

does not accumulate. Although these relationships have been elucidated only in the liver cells, they likely occur also in cell-types of other organs.

2. Homeostatic Mechanisms

Attention also should be paid to the homeostatic mechanisms of humans and experimental animals that may be important with respect to disease outcomes. The stage of the cell cycle, level of differentiation, and location in the body all affect a tissue's response to toxic insults. Somatic cell mutations, induced in the cells of a tissue with great regenerative potential, may ultimately compromise the health of exposed individuals more severely than if the toxic insult had resulted in cell death. For instance, mutagenesis of pluripotent stem cell lines or committed cell lines of the bone marrow may result in the appearance of late onset diseases such as myeloid disorders (e.g., anemias, polycythemias), myeloproliferative and lymphoproliferative disorders (e.g., leukemias and cyclic neutropenia), and irregular immunological processes (e.g., cytopenia, immune complex disease, toxic epidermal necrolyis, lymphadenopathy, and autoimmune diseases).

C. NEED FOR COORDINATION OF EFFORTS

To ensure maximum efficiency in collecting relevant data, it is desirable to search systematically for information on existing efforts related to genetic risk assessment rather than to attempt to set up overlapping independent studies. Although effective collaboration among agencies is still sporadic, there are some promising developments. For example, the coordination between the U.S. Environmental Protection Agency and the National Toxicology Program (NTP) to obtain dosimetric information on chemicals being tested in the mouse specific locus tests should enable existing NTP studies to better be used for purposes

of genetic risk assessment. It now seems appropriate that an oversight committee be formed for guidance on needs in human biomonitoring and to facilitate the coordination of efforts in genetic risk assessment.

The research effort required to answer major questions in genetic risk assessment must be cumulative rather than episodic, and funding for these efforts should endure over many years. With proper support, an oversight committee could help to ensure that this is accomplished. Investigators studying exposure or genotoxic damage in different tissues and different organisms must somehow integrate and focus their efforts. More effective sharing of valuable materials would certainly be useful in this respect. Within the purview of an oversight committee, a repository for biological materials obtained from animals and humans that have been exposed to putative mutagens should be established.

A series of regularly scheduled workshops should be inaugurated to facilitate collaboration. These workshops should include investigators using the biological materials in the repository and should provide for a cumulative review, comparison of results, and the identification of research needs.

It may be worthwhile to consider the selection of a few key compounds for concentrated, long-term efforts in genetic risk assessment. Since a long-term effort to collect data on chemical mutagenesis in human germ cells is a major undertaking, with important implications for genetic risk assessment, it should not be undertaken lightly. There are other issues that could be considered by an oversight committee.

The scientific and administrative issues in genetic risk assessment are too complex to be resolved here, and consequently, follow-up efforts are warranted. This entails coordinating efforts among the Federal agencies and among government agencies, industrial concerns, and the academic community.

Some preliminary efforts are underway, and it is hoped that these efforts will be expanded.

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